

ACTIVATION OF CUMINGIA AND ARBACIA EGGS BY BIVALENT CATIONS¹

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The activation of unfertilized eggs by isotonic salt solutions was first described by R. S. Lillie (1910, 1911*a*, 1911*b*). Since then, the activation by isotonic salt solutions of the eggs of seven marine invertebrates, belonging to three different orders, has been reported; *Pomatoceros* by Hörstadius (1923), *Asterias* by Dalcq (1924*a*, 1924*b*), *Phascolion* by Pasteels (1935), *Hydroides* by Pasteels (1935), *Barnca* by Dalcq (1928), *Thalassema* by Hobson (1928) and *Nereis* by Spek (1930).

The present work is a study of the effects of isotonic solutions of CaCl_2 , BaCl_2 , SrCl_2 , MgCl_2 , NaCl , KCl and LiCl , singly and in varying binary mixtures and proportions, on the eggs of *Cumingia tellinoides* and *Arbacia punctulata*; of the relative effectiveness of CaCl_2 in the activation of ovary eggs and shed eggs and of shed eggs that have been washed and shed eggs that have not been washed; and of the relative effectiveness of solutions of isotonic CaCl_2 which vary in pH. While its principal contribution is an extension of our knowledge of the effects of various isotonic salt solutions in the activation of eggs, it is hoped that it may illuminate further our understanding of the fundamental reaction or series of reactions which underlie the vital response of the cell.

Many careful investigators have shown that various types of stimulation cause an increase in permeability of the plasma membrane of various kinds of living material. However, Heilbrunn (1937) points out "There is one type of stimulation which can scarcely be conceived of as producing an increase in permeability. This is the stimulation produced by calcium salts. Students of permeability are quite unanimous in regarding the calcium ion as a permeability lowerer rather than a permeability increaser. Hence the action of calcium in producing stimulation cannot be explained on the basis of the permeability theory." Heilbrunn and his students have developed a colloid chemical theory of stimulation in which calcium plays the dominant rôle (see Heilbrunn, 1928; Heil-

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brunn and R. A. Young, 1930; Heilbrunn and Daugherty, 1933; Heilbrunn and Mazia, 1936; Angerer, 1936; Mazia and Clark, 1936; Heilbrunn and Wilbur, 1937; Donnellon, 1938). This theory postulates the following series of changes: calcium is released from the cortex resulting in a liquefaction of the cortex; free calcium enters the interior of the cell; as the concentration of free calcium increases in the cell interior, a series of reactions is initiated which includes an initial decrease in viscosity followed by a characteristic clotting reaction. This series of reactions constitutes the vital response of the cell. My observations on the activating effect of bivalent cations appear to support the colloid chemical theory of stimulation as developed by Heilbrunn and his students.²

MATERIAL AND METHODS

In most of the experiments the same general procedure was employed. In any given comparison the eggs from one female were used. The eggs were shed into sea water, the supernatant fluid withdrawn and two drops of a dense suspension of eggs were quickly pipetted into dishes of experimental solutions previously prepared. No attempt was made to control the temperature of the experimental solutions. The temperature of the air was no higher than 26° C. at any time and was usually between 21° and 25°. In any given experiment the range was rarely more than 2° C.

Merck's C.P. chemicals were used in making the solutions. NaCl, KCl and LiCl were made up in 0.53 M concentration and MgCl₂, BaCl₂, CaCl₂ and SrCl₂ in 0.3 M concentration. These solutions are isotonic with sea water and the eggs do not shrink or swell in them. In studying the effects of various mixtures of isotonic salt solutions in the activation of eggs, the pH of the various solutions was adjusted, by the addition of 0.1 N HCl and 0.1 N NaOH, so as to lie in the range found experimentally to be most favorable for activation, i.e. pH 6.2 to 8.6 for *Cuumingia* eggs and pH 8.8 to 9.0 for *Arbacia* eggs.

Eggs were exposed for varying periods of time to isotonic solutions of a single chloride or to mixtures of chlorides in varying proportions. The eggs were not transferred to sea water as is the usual procedure in experiments of this kind, inasmuch as a high percentage of cleavage could be obtained in the experimental solutions. In each experiment, hundreds of eggs were examined and 100 eggs were counted. The time factor is very important in determining the percentage of cleavage. At the end of

² This work was done at the Marine Biological Laboratory at Woods Hole during the summers of 1935, 1936 and 1937.

The problem was suggested by Dr. L. V. Heilbrunn. I wish to express my appreciation for his invaluable guidance and kind criticism during the course of this investigation.

The complete data are on file in the Library of the University of Pennsylvania.

a certain period, which is roughly three hours in the case of *Cumingia* eggs and five hours in the case of *Arbacia* eggs, there is no further increase in the percentage of cleavage and cell injury occurs a little later. It is desirable to count the percentage of activation at the end of this optimum period which varies with the solution, the pH and the temperature.

Conclusions concerning the effectiveness of a reagent in activating eggs are based on the percentage of cleavage. Although the first visible sign of activation of *Cumingia* eggs is the extrusion of polar bodies, it is difficult to make an accurate count of the percentage of eggs with polar bodies, for if the egg lies with the animal pole down, the polar bodies cannot be seen. While the first sign of activation when *Arbacia* eggs are inseminated is the elevation of the vitelline membrane, this reaction cannot be employed with isotonic solutions since they do not cause membrane elevation although they do cause the membrane to swell.

TABLE I

Experiments were performed to determine the relative effectiveness of barium, calcium and strontium solutions on the eggs of 33 individuals and tables were prepared of the percentage of cleavage and polar body formation and of the number of minutes elapsing before polar body formation.⁴ The following results were obtained.

Activated by	Average time of pb formation	Average percentage cl	Average percentage pb
barium	11 min. 48 sec.	5.3	3.6
calcium	11 min. 27 sec.	35.4	26.1
strontium	10 min. 54 sec.	35.8	38.2
sperm	10 min. 6 sec.		

RESULTS

Cumingia

Effect of 0.3 M CaCl₂.—When unfertilized *Cumingia* eggs are placed in 0.3 M CaCl₂, at any pH between 6.0 and 8.6, the first polar body is extruded in from 5 to 12 minutes and the first cleavage is completed in from 40 to 60 minutes. This is approximately the same as the time of polar body formation and of cleavage in eggs activated by sperm. The percentage of cleavage at the end of several hours varies widely among the eggs of different individuals. In some individuals 100 per cent of the eggs undergo apparently normal activation. They continue to cleave for several hours, reach the 8–16 cell stage and appear to be healthy and normal. After several hours, however, the blastomeres pinch in and fall apart.² Polar body formation is extremely irregular, but there appears

to be an inverse relationship between the percentage of polar body formation and the percentage of cleavage (compare Morris, 1917).

The result of experiments in which eggs were exposed to solutions of CaCl_2 which vary in pH from 3.6 to 9.0 is shown in Table I. The various solutions from pH 6.0 to 8.6 are equally effective in inducing activation of the eggs of most individuals. The pH of the solutions appears to have more effect on the percentage of cleavage than on the percentage of polar body formation, polar body formation proceeding at pH 9.0 while the percentage of cleavage decreases above pH 8.6.³ Both are almost completely inhibited at pH 4.1. (See Table II.) While the

TABLE II

Effect of pH on Activation of *Cumingia* Eggs by Isotonic Calcium Chloride

No. of exp. Time of Hrs. expos. Min. Temp. °C.	1 4 20 21.3 pb cl	2 4 55 21.3 pb cl	3 4 15 21.3 pb cl	4 6 20 20.0 pb cl	5 6 20 20.0 pb cl	6 7 15 20.0 pb cl	7 4 20 21.3 pb cl	8 4 55 21.3 pb cl	9 6 55 20.0 pb cl	10 7 15 20.0 pb cl
pH										
4.1	0 0	3 1	0 0			0 0	0 0	0 0		
4.6				60 5	32 0	19 0	0 0		13 0	40 0
4.9	55 2	34 18	21 12				14 38	9 27		
5.8				13 70	40 13	60 2		7 70	18 1	35 2
6.1	13 45	16 51	20 19				16 50			
6.2				2 98	6 98	4 94			30 5	8 10
7.1				2 98	6 88	6 91			25 5	2 14
7.6	22 45	10 63	30 32	2 98	5 90	9 89	16 55	8 66	56 3	3 15
8.2	18 49	14 59	11 54	32 92			14 59	4 65		
8.3				2 96	6 92	6 90			52 0	6 26
8.6	19 60	18 50	20 55	13 87	11 88	10 77	19 60	10 50	35 1	12 27
9.0	17 33	24 22	6 52	34 22	27 67	27 67	17 33	12 55	20 3	13 18
sw	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

strontium solution is somewhat more effective than the calcium solution, the former causes the blastomeres to separate in less time. It is difficult to make accurate counts of the percentage of cleavage when blastomeres separate.

Effect of Mixtures of Calcium Chloride and Some Monovalent Cations.—The result of experiments in which eggs were exposed to mixtures of potassium chloride, sodium chloride or sea water and calcium chloride in various proportions is shown in Fig. 1. The degree of activation decreases rapidly as the proportion of potassium increases up to a ratio of 1-16 and then remains fairly constant. It may be concluded that there is an antagonism of calcium by potassium between ratios 1-64

³ This work is not a study in artificial parthenogenesis and no attempt was made to develop procedures for securing later stages of development.

and 1-16 since the percentage of activation decreases too rapidly to be due to the dilution of calcium ions by potassium ions. Sodium has only a slight inhibiting influence on the activating effect of calcium from ratio 1-64 to ratio 1-4, but inhibition increases markedly above an average ratio of 1-4. The slight decrease in the percentage of activation up to an average ratio of 1-4 is probably due to the dilution of the calcium

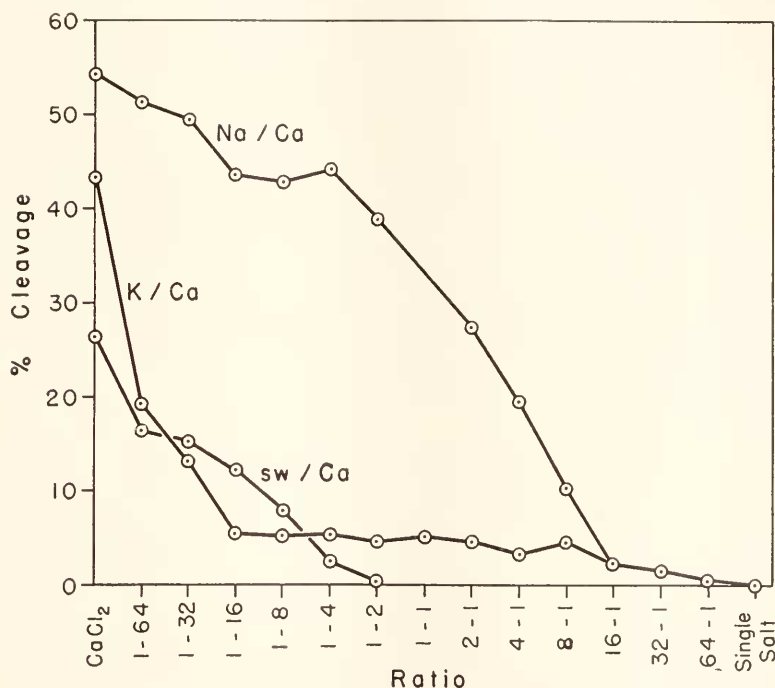


FIG. 1. Action on *Cumingia* eggs of isotonic solutions of some monovalent cations and of CaCl₂ singly and in various mixtures and proportions.

K/Ca—average of ten experiments.

Na/Ca—average of sixteen experiments.

sea water/Ca—average of five experiments.

solution, but there is an antagonism of calcium by sodium above this ratio. Sea water has an inhibiting effect on the activating effect of calcium beginning in a ratio of 1-64 and increasing as the proportion of sea water increases, with complete inhibition in most cases at a ratio of about 1-2. The inhibiting effect of sea water is no doubt due to the monovalent cations.

Effect of Mixtures of Calcium Chloride and of Some Bivalent Cations.—The result of experiments in which eggs were exposed to mixtures of strontium, barium or magnesium and calcium in various propor-

tions is shown in Fig. 2. Strontium is somewhat more effective than calcium in inducing cleavage in *Cumingia* eggs but causes the polar bodies to be extruded far from the cell surface and the blastomeres to separate in a short time. Strontium inhibits slightly the activating effect of calcium while calcium inhibits somewhat the activating effect of strontium. Solutions in which the Sr/Ca ratios are from 1-1 to about 64-1 are more injurious than the single salt solutions and it is difficult to make an accu-

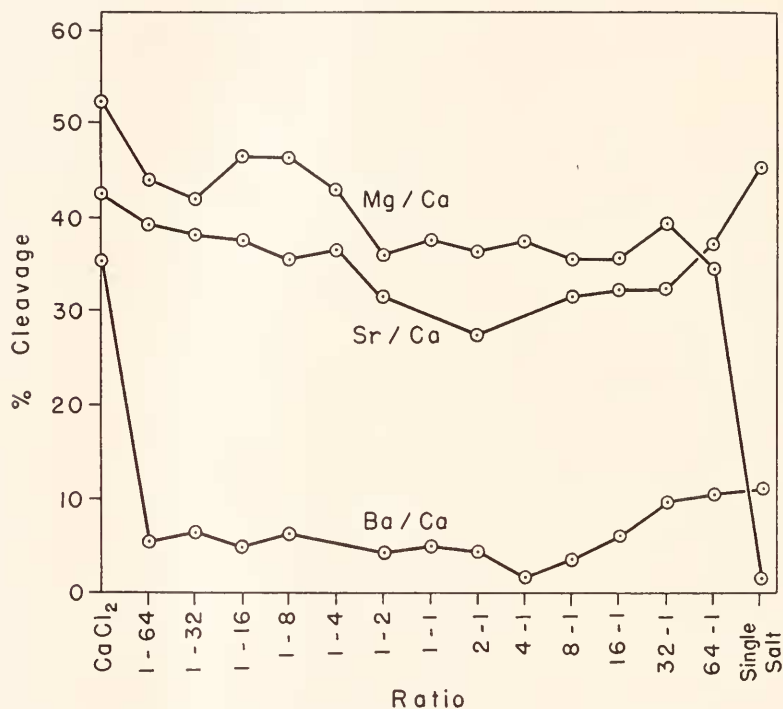


FIG. 2. Action on *Cumingia* eggs of isotonic solutions of some bivalent cations singly and in various mixtures and proportions.

Sr/Ca—average of seventeen experiments.

Ba/Ca—average of twelve experiments.

Mg/Ca—average of thirteen experiments.

rate count of the percentage of cleavage between these ratios. Barium has a slight activating effect on *Cumingia* eggs. It may be concluded that there is an antagonism of calcium by barium as the latter produces a marked inhibition of the activating effect of calcium, beginning in a ratio of 1-64. The percentage of activation resulting from exposing eggs to isotonic MgCl₂ is negligible. Magnesium inhibits very slightly the activating effect of calcium even in a ratio of 64-1. Inasmuch as magnesium has no activating effect on *Cumingia* eggs, it might be ex-

pected that as the dilution of the calcium solution by magnesium increases, the percentage of activation would decrease. It is interesting and noteworthy that this is not the case. Even in an Mg/Ca ratio of 64-1 there is a high percentage of cleavage.

In summary, monovalent cations are not effective in activating the eggs of *Cumingia* and they inhibit the activating effect of calcium, the effect increasing as the K/Ca, Na/Ca, sea/Ca ratios increase. The bivalent cations, with the exception of magnesium, are able to activate *Cumingia* eggs and in certain combinations and proportions, mutually inhibit activation. There is considerable variation in the behavior of different lots of eggs, but it is a variation in magnitude rather than in kind. The results of experiments performed on the eggs of a single individual (see original manuscript) are more interesting than the average of the results of many experiments as presented in this paper, because the former presents a more characteristic picture of the behavior of marine eggs.

Arbacia

Effect of 0.3 M CaCl₂.—When unfertilized *Arbacia* eggs were placed in 0.3 M CaCl₂, at any pH between 8.0 and 8.5, a certain percentage (rarely more than 25 per cent) of the eggs of most individuals undergo cleavage. It is difficult to make reliable counts of the percentage of activation in *Arbacia* eggs inasmuch as cells that have undergone cleavage usually occur in groups. The time required for maximum percentage of cleavage is from 7 to 10 hours or considerably longer than the time of cleavage in eggs activated by sperm. In a study of the eggs of 40 individuals the percentage of cytolysis was found to be high (33.5 per cent) if the eggs were aged for about 11 hours before being placed in the calcium solution.

A comparative study was made of the percentage of cleavage when the eggs were obtained in various ways. In some instances a fragment of ovary was placed directly in the solution to be tested. The exuding eggs are called ovary eggs. If such exuded eggs were washed in sea water they are called washed eggs. Shed eggs were obtained in the usual manner. Experiments were performed on the eggs of about 60 individuals and tables were prepared on the comparative percentage of cleavage. These tables are elaborate and it was thought unwise to attempt their publication (see original paper). The following results were obtained:

Average percentage of cleavage

Ovary eggs.....	11.8
Shed eggs.....	22.8
Shed eggs.....	23.8
Washed shed eggs.....	30.9

There is a higher percentage of cleavage in shed eggs than in ovary eggs and a slightly higher percentage of cleavage in washed shed eggs than in shed eggs.

Table III shows that when the unfertilized eggs of one individual are placed in solutions of CaCl_2 in which the pHs vary from 3.8 to 9.6, for from 5 to 9 hours, the percentage of cleavage is low below pH 8.0 and is only slightly higher at pH 8.5 while the highest percentage of cleavage takes place at about pH 9.0. Cleavage takes place in the short-

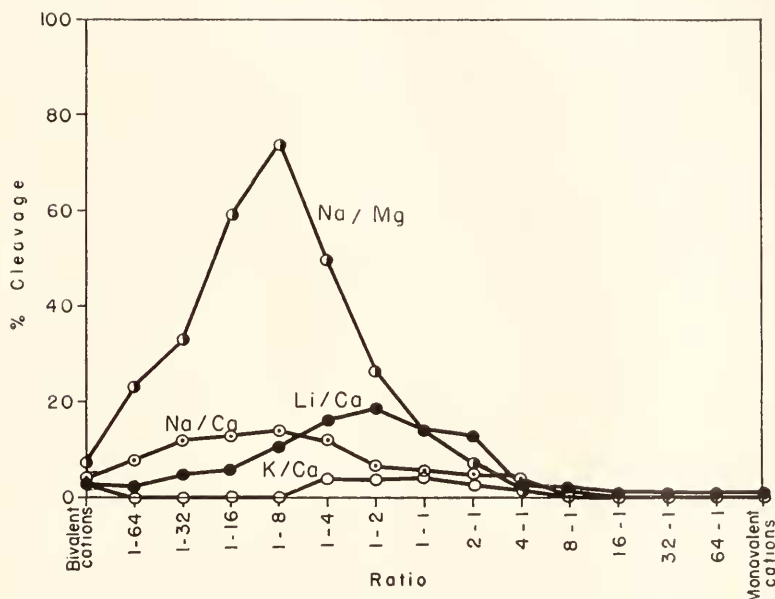


FIG. 3. Action on *Arbacia* eggs of isotonic solutions of some monovalent and bivalent cations singly and in various mixtures and proportions.

K/Ca—average of fourteen experiments.

Na/Ca—average of nine experiments.

Li/Ca—average of eight experiments.

Na/Mg—average of three experiments.

est time at pH 9.2 but the eggs soon undergo cytolysis. High alkalinity also seems to cause nuclear division without cytoplasmic cleavage in a large percentage of eggs. In many cases there is little and sometimes no cleavage in solutions of which the pH is about 8.8 while there may be a high percentage of cleavage in solutions of which the pH is about 9.0. *Arbacia* eggs are not activated by alkaline sea water.

Effect of Mixtures of Calcium Chloride and Some Monovalent Cations.—The result of experiments in which eggs were exposed to solutions of potassium, sodium or lithium and calcium chloride in various

proportions and of sodium and magnesium chloride in various proportions is shown in Fig. 3. There is a gradual increase in the percentage of activation as the proportion of potassium increases up to a ratio of about 1-2 while above this ratio there is a decrease with activation ceasing at a ratio of about 8-1. From ratios 16-1 to 64-1 there are many eggs in which the nucleus has undergone several divisions and in which

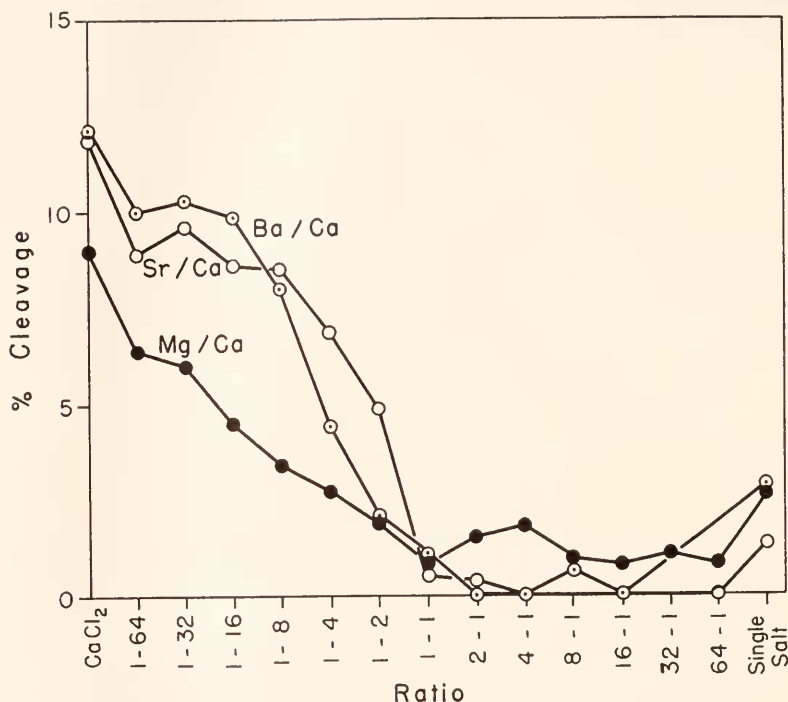


FIG. 4. Action on *Arbacia* eggs of isotonic solutions of some bivalent cations singly and in various mixtures and proportions.

Mg/Ca—average of eight experiments.

Sr/Ca—average of eight experiments.

Ba/Ca—average of eleven experiments.

there has been no cytoplasmic division. There is a marked increase in the percentage of activation as the proportion of sodium increases up to about ratio 1-4 followed by a marked decrease, with no activation in mixtures in which the ratio is above 16-1 or in the Na solution. Isotonic LiCl solution is able to activate a small percentage of *Arbacia* eggs. The degree of activation increases rapidly as the proportion of lithium increases beginning in a ratio of about 1-16, with the highest percentage at a ratio of about 1-2, followed by a sharp decline at a ratio of about

2-1. Isotonic $MgCl_2$ is able to activate a very small percentage of eggs. There is a very great increase in the percentage of activation as the proportion of sodium increases up to an average ratio of 1-8 followed by an equally sudden decrease with activation ceasing at a ratio of about 8-1. This result is of interest because there is a marked increase in the percentage of activation both in Na/Ca mixtures and Na/Mg mixtures between ratios of about 1-32 and 1-8.

Effect of Mixtures of Calcium Chloride and Some Bivalent Cations.

—The result of experiments in which *Arbacia* eggs are exposed to solutions of magnesium, strontium or barium chloride and calcium chloride in various proportions is shown in Fig. 4. While magnesium has a slight activating effect, there is a mutual antagonism between calcium and magnesium. Strontium is able to activate a small percentage of eggs but is much less effective than calcium. The degree of activation by calcium decreases gradually as the proportion of strontium increases up to a ratio of about 1-8 with a sharp decrease above a ratio of 1-8. It may be concluded that there is an antagonism of calcium by strontium. Barium alone is able to activate a small percentage of eggs. The degree of activation decreases as the proportion of barium increases with activation ceasing at a ratio of about 2-1. It may therefore be concluded that there is antagonism of calcium by barium. There are many eggs with nuclear divisions without cytoplasmic cleavage in mixtures where the Ba/Ca ratio is between 32-1 and 64-1.

In summary, Na and K are not effective in activating *Arbacia* eggs while Li activates a small percentage. The monovalent cations increase the percentage of activation by calcium when present in certain definite proportions. The bivalent cations, Sr, Ba and Mg each have an inhibiting effect on activation by Ca and the antagonism is mutual. There is even more variation in the behavior of *Arbacia* eggs than in *Cumingia* eggs but again it is a variation in magnitude rather than in kind (see original paper).

DISCUSSION

The problem of the activation of unfertilized eggs by an alteration of their chemical environment has been vigorously attacked by a number of investigators. R. S. Lillie (1910, 1911a, 1911b) was the first to report activation of marine eggs by means of isotonic salt solutions. To date, the activation by isotonic salt solutions of the eggs of seven species of marine invertebrates has been reported. In each case, activation was accomplished by exposing eggs to varying combinations and proportions of the chlorides of the cations of sea water. In all, the presence of Ca appears to be essential while there is variation in the element which it is necessary to add.

R. S. Lillie (1910) reported the initiation of development in *Arbacia* eggs when exposed to isotonic NaCl for varying periods of time followed by return to sea water. He reported no activation of *Arbacia* eggs when exposed to isotonic solutions of CaCl_2 , SrCl_2 and MgCl_2 followed by return to sea water. In the present work the opposite of these observations is reported. The difference in results obtained with the monovalent cations may be due to the fact that in the present work the eggs were not returned to sea water and the difference in results obtained with bivalent cations may be due to the fact that *Arbacia* eggs must be exposed to isotonic solutions of CaCl_2 , SrCl_2 and MgCl_2 for hours in order to obtain a noteworthy percentage of activation.

The results of the experiments on the eggs of *Arbacia* reported in this paper are in agreement with the results of the work on marine eggs reported by Dalcq (1928) on *Barnea candida*, Hobson (1928) on *Thalassema neptuni* and Pasteels (1935) on *Phascolion* and *Hydroides* where alkalinity and the monovalent cations K, Na and Li increase markedly the percentage of activation of *Arbacia* eggs by Ca and are necessary in order to obtain a high percentage of activation of the eggs of most individuals and where cleavage appears to be more nearly normal in favorable binary mixtures than in isotonic CaCl_2 alone.

However, the results on activation of the eggs of *Cumingia* by isotonic salt solutions are not in agreement with the results of the work reported by Dalcq, Hobson and Pasteels on activation of marine eggs by isotonic salt solutions. The addition to the calcium solution of excess OH ions or of the monovalent cations Na or K does not increase the percentage of activation of *Cumingia* eggs but has the opposite effect. The segmenting eggs appear more nearly normal and more healthy in isotonic CaCl_2 alone than in any of the binary mixtures used. Activation by isotonic CaCl_2 with respect to time of polar body formation and percentage of activation compares favorably with activation of eggs by sperm. We may say that in the case of *Cumingia* eggs, Ca is the sole activating agent and that no other external agent or treatment is necessary.

There are several theories to explain the activation of unfertilized eggs. All these theories are aspects of more general theories of stimulation. The oxidation theory of activation was stated by J. Loeb (1913). It is now quite certain that not all activating agents increase the rate of oxidation. Heilbrunn (1915) pointed out that cyanide does not prevent the first stages of development in *Arbacia* eggs and (1920a) that maturation in *Cumingia* eggs is not dependent on an increase in oxygen consumption. Whitaker (1931, 1932) reported that in the eggs of *Nereis* and *Arbacia* there is an increase in the rate of respiration following fertilization whereas in the eggs of *Chaetopterus* and *Cumingia* there is

a decrease following fertilization. Activation of eggs by an isotonic solution of CaCl_2 can scarcely be due to an increase in the rate of oxidation for calcium is usually thought to decrease the rate of oxidation (see for example, Ahlgren, 1925; Holek, 1934; and Thunberg, 1937). It may therefore be concluded that rate of oxygen consumption is not the primary factor in the initiation of development of eggs.

The permeability theory of activation, founded by R. S. Lillie (1916, 1917, 1918) has been used to explain initiation of development in eggs. That there is an increase in permeability in some marine eggs following activation has been convincingly demonstrated by a number of careful investigators. The work of Lillie (1916, 1917, 1918) and McCutcheon and Lucké (1932) shows that the permeability of *Arbacia* eggs to water increases after fertilization and the work of Stewart and Jacobs (1932) shows that permeability of these eggs to ethylene glycol increases after fertilization. However, activation of eggs by isotonic CaCl_2 cannot be conceived of as due to an increase in permeability. It is universally agreed among students of permeability that bivalent cations such as magnesium and calcium cause a decrease in cellular permeability and antagonize those reagents known to increase it. Therefore the action of calcium in the activation of *Cumingia* eggs cannot be explained on the basis of the permeability theory.

Dalcq (1924a, 1924b) has developed a depolarization theory of activation. This theory depends upon the presence of charges of definite sign upon the cortex and constituents of the egg and upon the existence of a potential gradient on the cortex. He concluded that a disturbance of the intraovular cations results in depolarization, that Ca is the most effective agent in bringing about depolarization and that activation is the result of depolarization. However, the depolarization theory of Dalcq seems highly speculative and is difficult to understand from the electrochemical standpoint.

Heilbrunn (1915) favored the coagulation theory of activation. This theory, which is now termed the colloid chemical theory is, as the permeability theory, a broad theory of stimulation for all types of irritable systems. In a study of the chemical changes in the egg following activation, Heilbrunn and his students have shown that whenever a cell is stimulated, Ca is released from the cortex. Heilbrunn and his students have further shown that if Ca is first removed from egg cells by oxalate, stimulating agents are not effective but that upon the return to sea water the usual response may be obtained (see Heilbrunn and R. A. Young, 1930; Heilbrunn and K. Wilbur, 1937). For a full discussion of the colloid chemical theory of stimulation see Heilbrunn's "Outline of General Physiology," 1937. The results of the study of the activa-

tion of the eggs of *Arbacia* by favorable binary mixtures of bivalent and monovalent cations and of the study of the activation of the eggs of *Cumingia* by isotonic solutions of bivalent cations alone, where 100 per cent of the eggs of some individuals undergo apparently normal cleavage in a period of time which compares favorably with the time of activation of eggs activated by sperm, favor the colloid chemical theory of Heilbrunn and are directly opposed to any interpretation in terms of the oxidation or permeability theories.

SUMMARY

1. When unfertilized *Cumingia* eggs are placed in 0.3 M CaCl_2 , 100 per cent of the eggs of some individuals undergo apparently normal cleavage. The time of polar body formation and of the first cleavage in eggs activated by Ca is approximately the same as the time of polar body formation and of cleavage in eggs activated by sperm.

2. Polar body formation and cleavage in *Cumingia* eggs proceed normally in 0.3 M CaCl_2 at the various pHs between pH 6.2 and pH 8.6 but are inhibited above and below this range.

3. The bivalent cations Sr, Ca and Ba are able to activate *Cumingia* eggs and are named in the order of their effectiveness.

4. The time of polar body formation in *Cumingia* eggs activated by isotonic solutions of SrCl_2 and BaCl_2 is approximately the same as the time of polar body formation in eggs activated by sperm.

5. The monovalent cations K and Na and sea water inhibit activation of *Cumingia* eggs by Ca. The percentage of activation decreases as the K/Ca, Na/Ca and sw/Ca ratios increase. K has a greater inhibiting effect than Na.

6. Ba inhibits the activation of *Cumingia* eggs by Ca, Sr inhibits very slightly the activating effect of Ca, while Mg does not appear to have an inhibiting effect on activation of eggs by Ca.

7. When unfertilized *Arbacia* eggs are placed in 0.3 M CaCl_2 , from 40 to 60 per cent of the eggs of most individuals undergo cleavage if the pH of the solution is between 8.8 and 9.2. No membrane is elevated in isotonic salt solutions.

8. Below pH 8.8 the percentage of cleavage is low and above pH 9.0 the percentage of cytolysis is high in *Arbacia* eggs activated by isotonic CaCl_2 .

9. Isotonic solutions of SrCl_2 , BaCl_2 and MgCl_2 are able to activate a certain percentage of *Arbacia* eggs, but these ions are not so effective as Ca and their action is somewhat variable.

10. The monovalent cations Na, Li and K in certain definite proportions increase the percentage of cleavage induced by Ca while in other

proportions they have the opposite effect. Similarly isotonic NaCl, in certain definite proportions increases markedly the percentage of activation by the Mg solution while in other proportions Na has the opposite effect.

11. Sr, Mg and Ba inhibit the activation of *Arbacia* eggs by Ca, the inhibiting effect increasing as the Sr/Ca, Mg/Ca and Ba/Ca ratios increase.

12. The results are brought into relation to the colloid chemical theory of stimulation.

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